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Gametophyte Morphology in Three Mexican Species of *Bolbitis* (Lomariopsidaceae)

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Abstract.—The development of gametophytes in *Bolbitis bernoullii*, *B. portoricensis* and *B. umbrosa* (Lomariopsidaceae) is described and compared. Spores are monolete, ellipsoid with prominent winged perispore. Germination is of the *Vittaria* type and the prothallial development is of the *Drynaria* type. Collenchyma-like thickenings at the corners of the wing cells were not observed. Adult gametophytes are cordate with scarce marginal hairs near the shallow notch. Gametangia are of the common type found in homosporous leptosporangiate ferns. *Bolbitis portoricensis* develops the first leaf of the sporophyte after 6 to 8 months, with polocytic stomata. In *B. portoricensis*, vegetative propagation is present in old thalli 6 to 8 months after cultivation.

The genus *Bolbitis* is a terrestrial or hemiepiphyitic fern, with dimorphic leaves and simple or pinnate blades. Some species have laminar buds, and all have fertile leaves with acrostichoid sori, and reticulate venation with or without included veinlets. Its spores are monolete, non-chlorophyllous, and winged (Mickel and Smith, 2004; Moran, 1995; Tryon and Tryon, 1982; Tryon and Lugardon, 1991). The genus contains about 45 species, a third of which are found in the New World; seven occur in the southeastern part of Mexico. These seven species grow between 0–1300 masl in very wet rainforests. Except for *B. portoricensis* (Spreng.) Hennipman, all species are uncommon or rare in Mexico.

The gametophyte morphologies are known for several Indian species: *Bolbitis semicordata* (Moore) Ching, *B. quoyana* (Gaud.) Ching, *B. subcrenata* (Hook. et Grev.) Ching in C. Chr. (Nayar, 1960; Nayar and Kaur, 1964a, 1964b, 1965a, 1965b, 1965c, 1971); and for Old Word species such as *B. angustipinna* (Hayata) Ito, *B. heteroclita* (Presl) Ching and *B. repanda* (Bl.) Schott (Hennipman 1970, 1977). In general terms, the mature prothallus of *Bolbitis* is cordate-thalloid, wider than long, with a dense, 6 to 8-celled thick cushion, and wide flat wings. Collenchyma-like thickenings are present in the corners of wing cells of some Indian species.

Gametophytes of *Bolbitis* can be morphologically different. For instance, the prothallus in *B. repanda* is cordate, with pluricellular branched hairs and, when old, has a lobed thallus. In *B. cladorrhizans* (Spreng.) Ching, the gametophyte is glabrous and ribbon-shaped or lobed. Other species such as *B. heteroclita* have cordiform-spatulate prothalli, with unicellular, secretory and papillate hairs sparsely distributed along the margin of the adult gametophyte. In species such as *B. semicordata, B. quoyana* and *B. subcrenata*, the adult cordate thallus is usually branched but may be un-branched and has club-

TABLE 1. Collection data for materials used in current study.

Scientific name	Collection No. and date	Site location	Habitat elevation
B. bernoullii	*AMR-843* 20/04/2004	Veracruz; Mun. San Andrés Tuxtla, Plot 69 at Biological Station Los Tuxtlas, UNAM, between Laguna Azul and Laguna Escondida.	SAP 20 m asl
B. portoricensis	*AMR-618* 03/05/2002	Veracruz; Mun. Catemaco, Peninsula Moreno, in direction on the river, ca. 14–15 km after Coyame, towards Adolfo López Mateos.	SAP 60 m asl
	AMR-848 20/04/2004	Veracruz; Mun. San Andrés Tuxtla, Plot 69 at Biological Station Los Tuxtlas, UNAM, between Laguna Azul and Laguna Escondida.	SAP 20 m asl
	AMR-857 22/04/2004	Oaxaca; Mun. Valle Nacional, 1 Km before San Mateo Yetla, in the direction of Valle Nacional, coming from La Esperanza.	BMM 120 m asl
	BPG-1189 21/04/2004	Veracruz; Mun. Catemaco, 6.5 Km after Tebanca, in direction of Hidalgo.	Remains of SAP 390 m asl
B. umbrosa	*BPG-1185* 21/04/2004	Veracruz; Mun. San Andrés Tuxtla, Biological Station of Los Tuxtlas, UNAM.	SAP 150 m asl

shaped, multicellular, marginal hairs, 2 to 6 cells long, with slender stalks and a swollen anterior region.

One year old, or older, gametophytes of *Bolbitis repanda* are broadly cordate or elongate, with brown marginal rhizoids, and are abundantly covered with simple glandular hairs. The gametangia are of the advanced type, as in all Lomariopsidaceae. The morphological variation of the gametophytes is not taxonomically significant, and offers no evidence for determining affinities to other genera of the Lomariopsidaceae (Stokey and Atkinson, 1957; Atkinson, 1973).

This paper describes and compares the morphology and development of three species in Mexico: *Bolbitis bernoullii* (Kuhn. ex H. Christ) Ching, *B. portoricensis* and *B. umbrosa* (Liebm.) Ching.

MATERIALS AND METHODS

Spores were obtained from live plants collected from several sites in Mexico (Table 1). Vouchers are deposited at the Herbario Metropolitano Ramón Riba y Nava Esparza (UAMIZ). Fertile pinnae of different individuals were placed in paper bags until the spores were released. To remove sporangia, a mesh with pores 0.074 mm in diameter was used to sieve them. The spores of each species were then sown in five Petri dishes with Thompson solution, mineral salts, and agar, at an average density of 100–150 spores per cm^2 (Mendoza-Ruiz and Pérez-García, 2003). The Petri dishes were placed inside transparent plastic bags to stop contamination and dehydration and placed under artificial sun light (75 Watts day-light lamps), a 12 h light/darkness photoperiod, and

a temperature of 23–25°C. To determine photoblastism, two dishes of each species were placed in the dark.

Results

Spores of all three species are monolete, ellipsoid, flat-convex laterally, oblong in the extremes (poles), with a short leasura, and a smooth exine. *Bolbitis bernoullii* spores average 39(42)49 µm long × 31(35)39 µm wide, *Bolbitis umbrosa* 37(39)42 × 29(31)34 µm, and *B. portoricensis* 37(39)42 × 30(32)35 µm. In all three, the perine is prominent, membranous, thin, with wrinkled undulate folds, and psilate. In *Bolbitis bernoullii* the perine measures 6(7)10 µm, in *B. umbrosa* 5(7)10 µm, and in *B. portoricensis* 4(5)7 µm (Figs. 1, 2 and 3).

Once the spore germinates, the exine ruptures from the leasura and the first rhizoid, which is hyaline and has scarce plastids, emerges. The first prothallial cell develops inside the spore wall and contains numerous chloroplasts and yellow, oily globules (Fig. 4). Germination begins between days 8 and 9 in *Bolbitis portoricensis*, and between days 14 and 22 in *B. umbrosa* and *B. bernoullii*. Spore germination is of the *Vittaria*-type (Mendoza, 2001) and results in a proximal rhizoid and, lateral to it, a uniseriate germinal filament with 2 to 6 short, barrel-shaped cells, each wider than long and densely chlorophyllous. This early phase takes place between days 9 and 22 in *B. bernoullii, B. portoricensis* and *B. umbrosa*; development across species is asynchronous (Figs. 5–8). After 100 days, none of the spores kept in the dark germinated.

In *Bolbitis portoricensis* and *B. bernoullii*, the development of a prothallial lamina starts with repeated longitudinal and transverse divisions of the antepenultimate cells of the filament, and, with the expansion of the resulting daughter cells, gives rise to a 7- to 14-celled plate phase with an apical, obconic meristematic cell on day 22 (Figs. 9–10). Filaments in *B. umbrosa* are short and immediately undergo longitudinal and transverse divisions that initiate the 5- to 7-celled, short, wide and cordiform-looking plate phase on day 22; the obconic meristematic cell in these plates is not clearly defined.

The prothallial lamina of *Bolbitis portoricensis*, from day 16 to 38, is cordate-spatulate, with 20 to 120 cells, has a central pluricellular meristem, and is glabrous (Figs. 11–12). Rhizoids are located at the base of the gametophyte (Figs. 13–14). In *B. bernoullii* (Fig. 15) and *B. umbrosa*, the laminar phase is widely cordate, glabrous, of ca 60 to 70 cells, and has a centrally positioned meristematic zone in the shallow apical notch; rhizoids appear on day 37 and are located along the central part of the gametophyte.

Prothallial development is of the *Drynaria*-type (Nayar and Kaur, 1969); the establishment of a meristematic cell occurs when the prothallial lamina is 7 to 14 cells wide. In some cases, a meristematic cell stage is absent and, a pluricellular meristem develops from the anterior marginal cells. A characteristic feature of this type of development is the much slower establishment of the apical meristem cell and the development of hairs on


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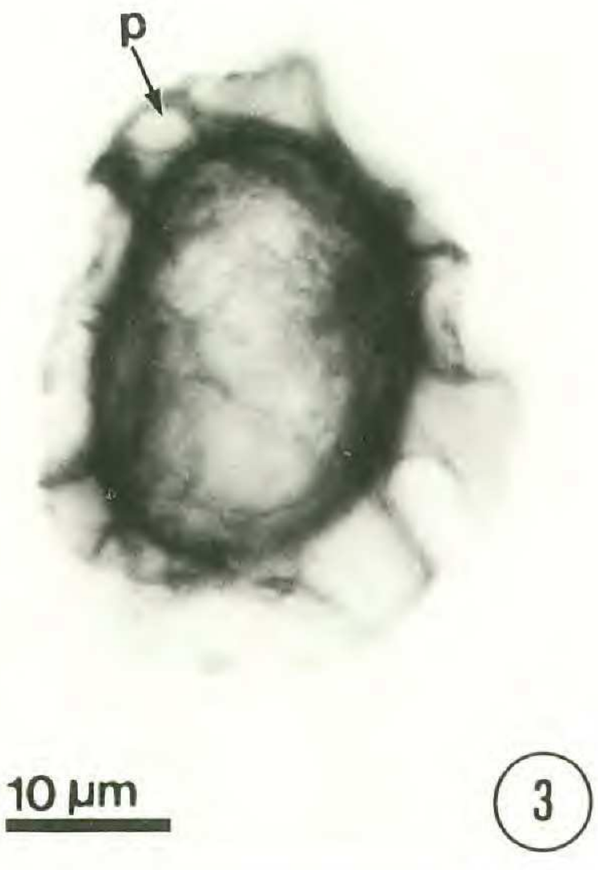
1


p
10 µm
2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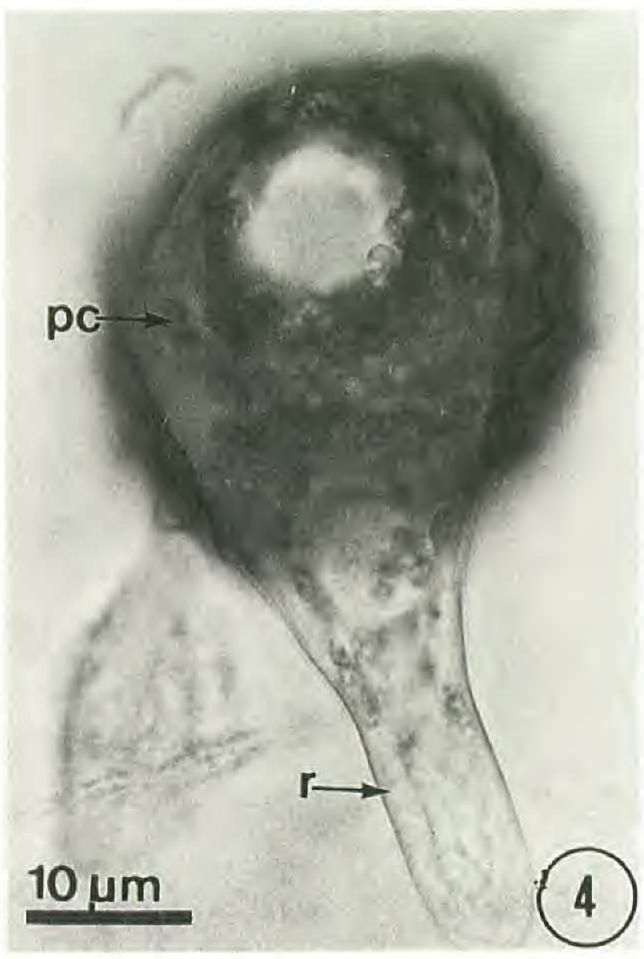
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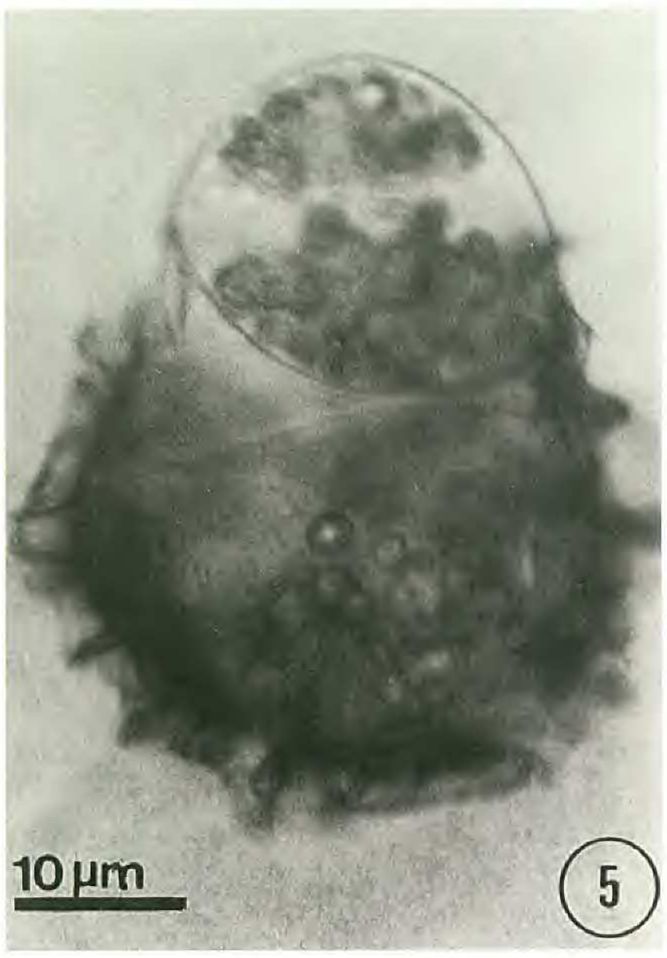
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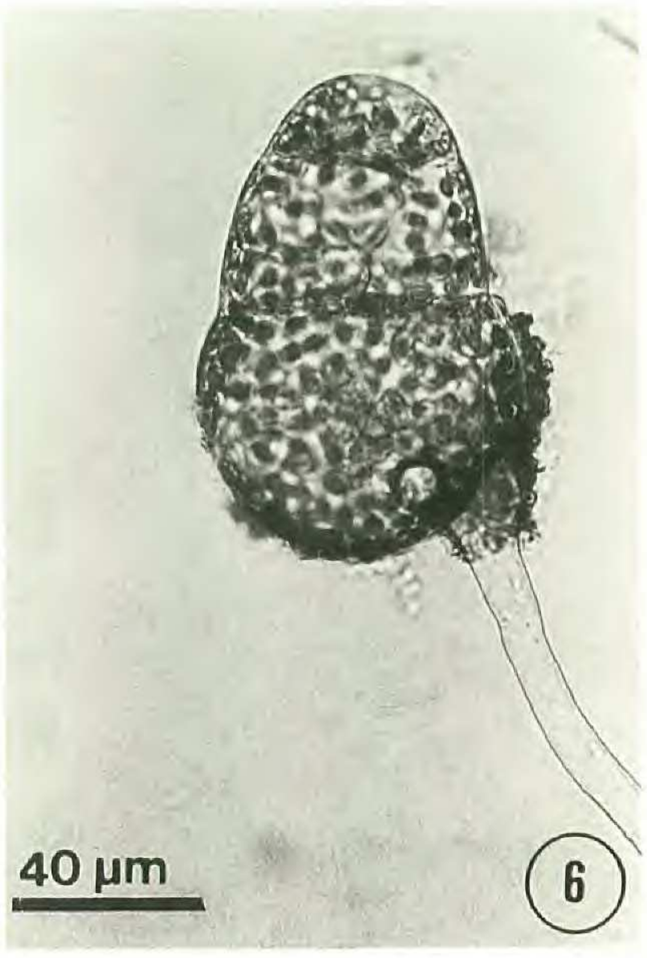
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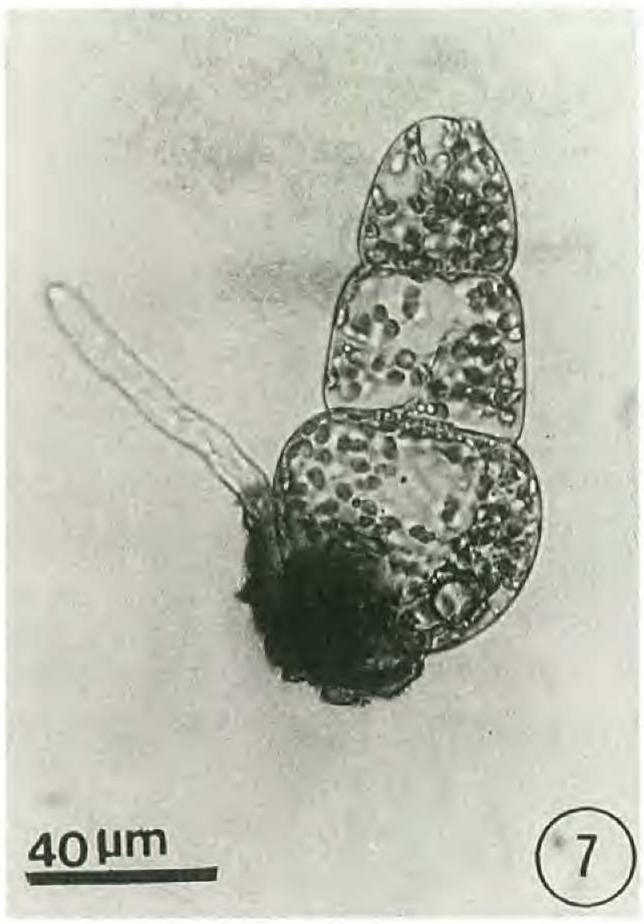
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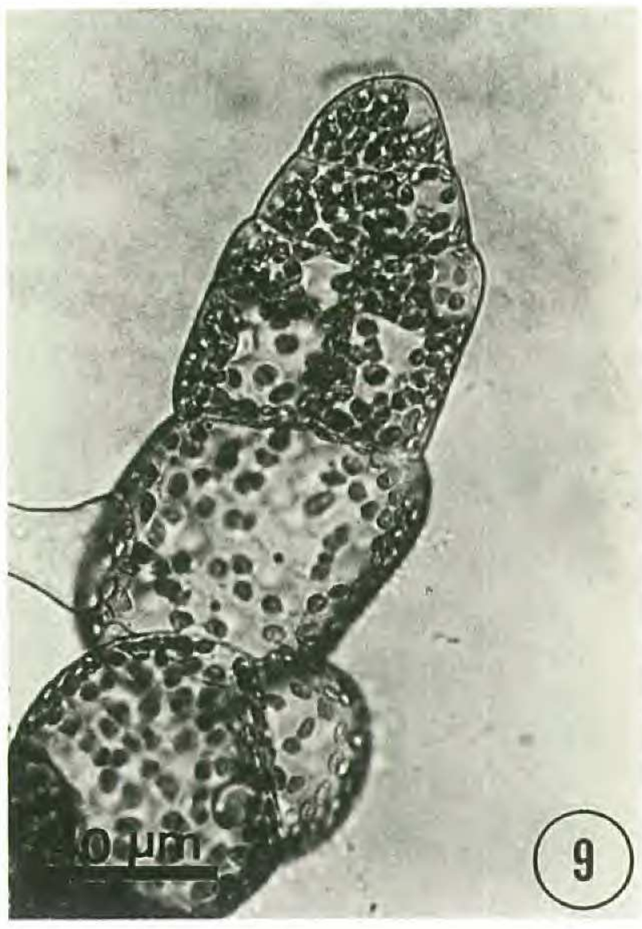
40 µm
9

the margin of the prothallus. In our species, the adult stages show very sparse marginal hairs near the shallow notch.

There are slight variations in the shape of the vegetative thallus. In *Bolbitis portoricensis* (day 37 to 50) they are cordate-spatulate with an inconspicuous meristematic zone and weakly defined wings; others are cordate, with a slightly deep to very deep apical notch, and isodiametric wings; still others are spatulate with a shallow meristematic area and more or less isodiametric wings. All gametophytes are nearly glabrous, with very scarce marginal hairs near the shallow notch. Rhizoids are located on the central basal region (Figs. 13, 14, 17). In *B. umbrosa* (day 50) gametophytes are cordiform with poorly defined wings and meristem and, in some cases, with very scarce marginal hairs near the apex. By day 79 they are cordate with a deep pluricellular meristem and isodiametric wings (Fig. 16).

In *Bolbitis bernoullii* the adult thallus is cordate-spatulate with more or less isodiametric and thin wings, a pluricellular meristem located in a small notch, abundant rhizoids in the basal region, sparse marginal hairs by days 65 to 75, and antheridia randomly distributed over the lamina (Figs. 5, 15, 23).

Adult gametophytes of *Bolbitis umbrosa* reach maturity between days 70 and 80. They are spatulate, with undefined wings and an apical, pluricellular meristem, have naked antheridia and abundant basal rhizoids (Fig. 24). In *B. portoricensis* there is variation in the adult phase: they can be cordiform-spatulate with isodiametric wings (day 37–62), cordiform-reniforme with wide wings and an undefined apical meristem (day 50–78), or cordiform-spatulate with short, isodiametric wings, a well defined pluricellular meristem, a dense cushion with antheridia, and smooth to slightly undulate margins (day 50 to 78) (Figs. 19, 22, 25). Laminar, bifurcate gametophytes, each bifurcation with its own apical meristem, commonly develop in cultures of *B. portoricensis* (Fig. 18).

Bolbitis portoricensis and *B. umbrosa* develop a 6- to 8-celled thick, dense cushion on which the sexual organs are located. The more or less centrally located rhizoids are usually restricted to the lower surface of the cushion, and are frequently found in clusters. Rhizoids are thin and hyaline except in *B. bernoullii* where the cushion is not well defined. No collenchyma-like thickenings were seen in our species.

Sparse marginal hairs develop near the meristematic zone of mature thalli in *Bolbitis portoricensis* after the development of the cushion (Figs. 19–21). The hairs consist of 2 or 4 cells and vary in frecuency. Rhizoids in *B. bernoullii, B. portoricensis* and *B. umbrosa* are unicellular extensions of the superficial cells. They have brown cells walls, with thin, long and tangled chloroplasts.

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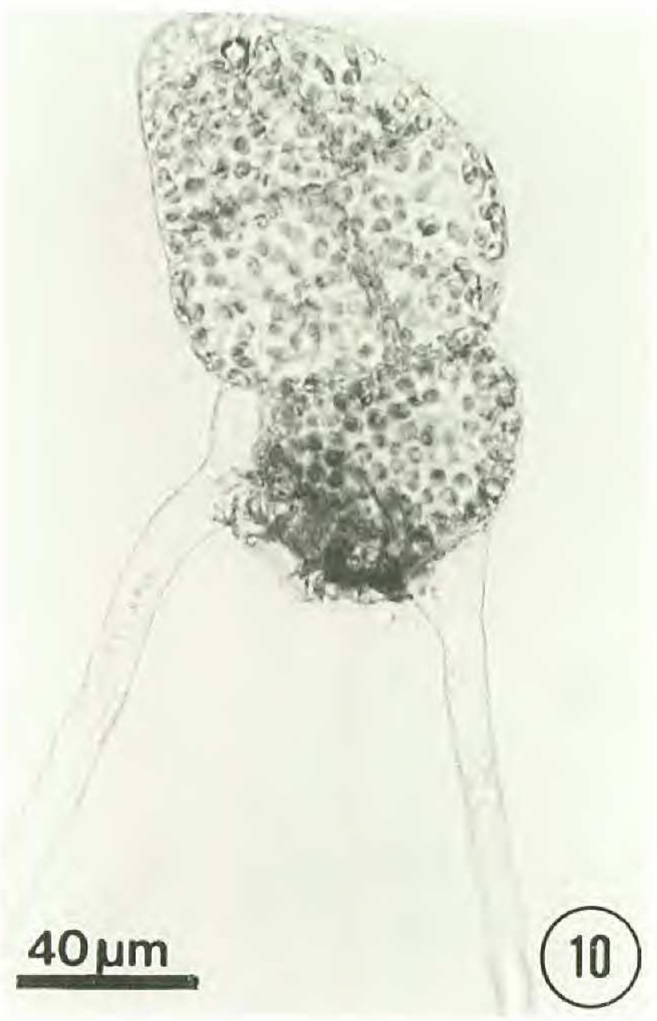
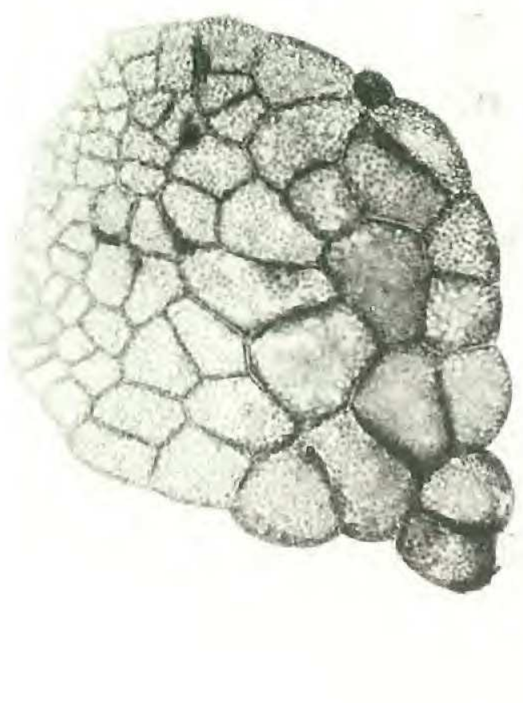
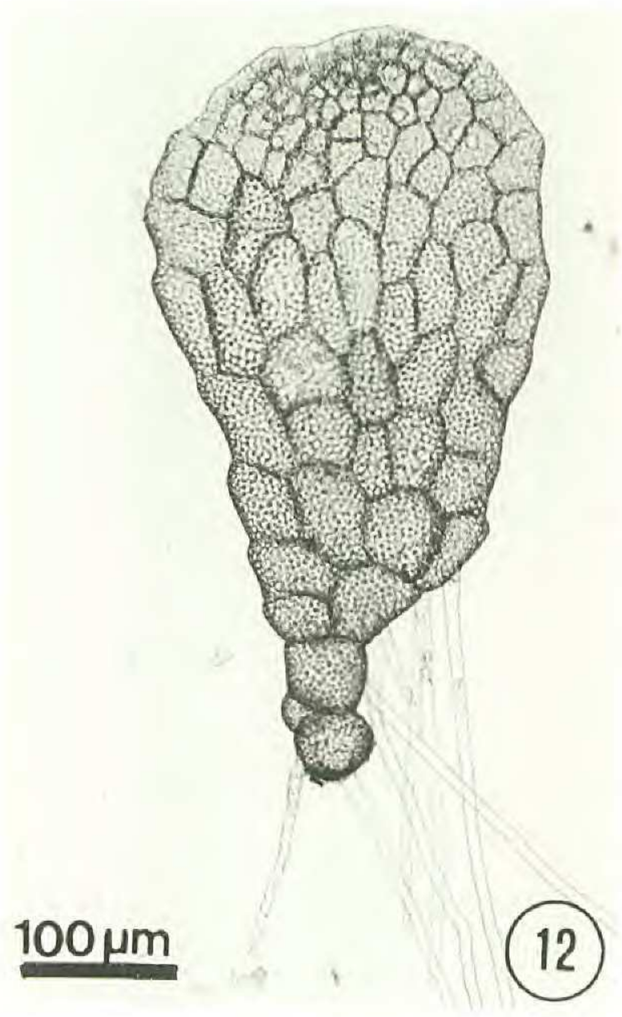
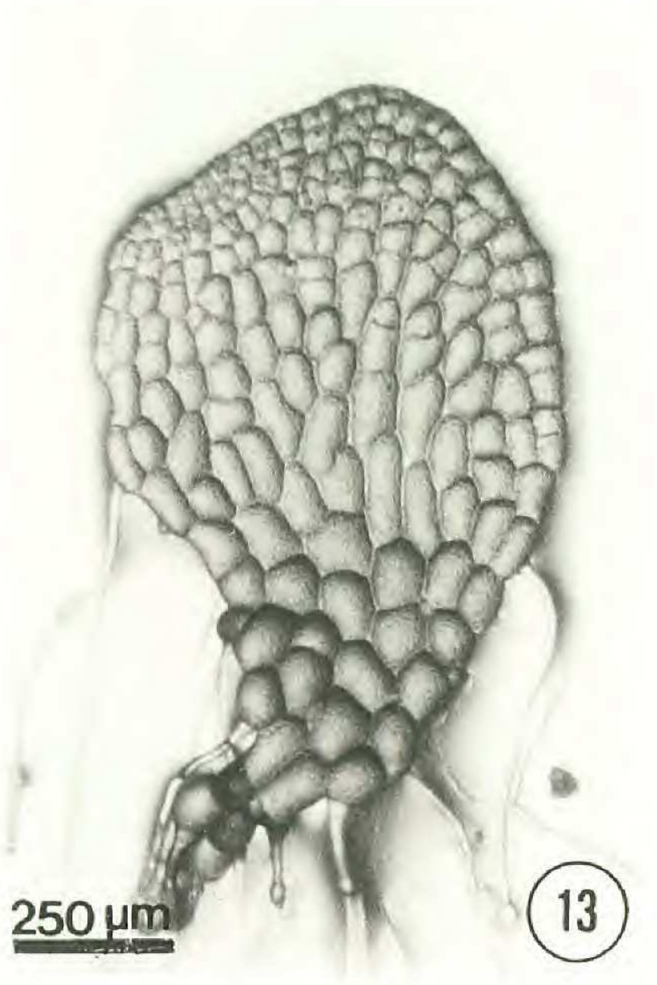
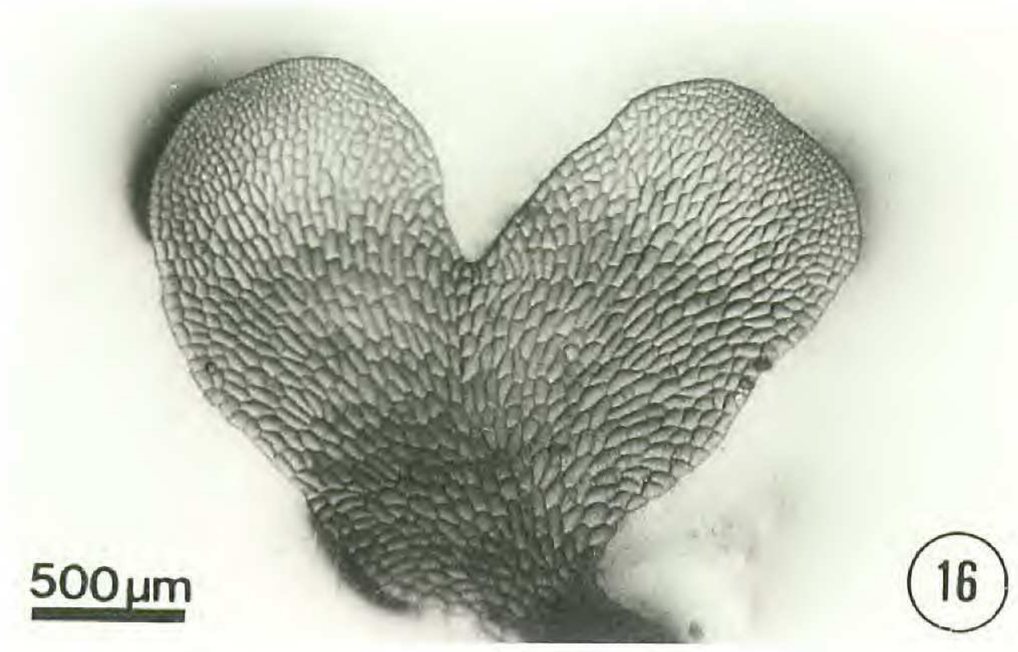
FIGS. 1–9. Spore germination and filamentous phases in *Bolbitis*. 1. Spore of *B. bernoullii*. 2. Spore of *B. portoricensis*. 3. Spore of *B. umbrosa*. 4. Germination in *B. umbrosa*, 8 days. 5. Two-celled filament in *B. bernoullii*, 20 days. 6. Three-celled filament in *B. umbrosa*, 16 days. 7. Three-celled filament in *B. portoricensis*, 9 days. 8. Six-celled filament in *B. portoricensis*, 15 days. 9. Beginning of the laminar phase in *B. portoricensis*, 16–22 days. p= perine, pc= prothallial cell, r=rhizoid.

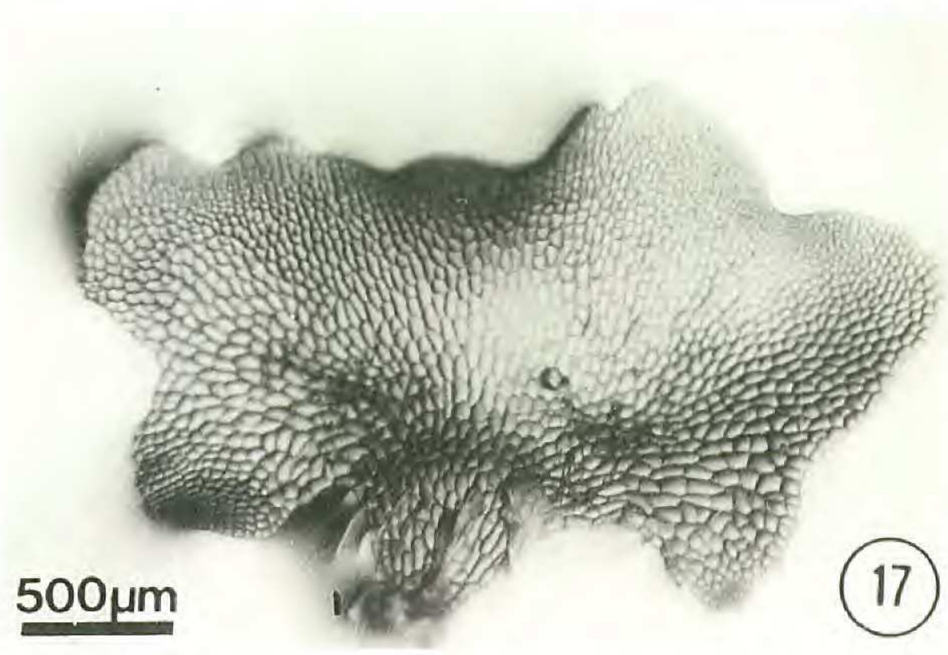












FIGS. 10–17. Young plate phases of *Bolbitis*. 10–12. Beginnings of the laminar phase in *B. umbrosa*, 16–37 days. 13–14. Laminar phases in *B. portoricensis*, 44–58 days. 15. Laminar gametophyte in *B. bernoullii*, 65–71 days. 16. Cordiform gametophyte with a deep notch in *B. umbrosa*, 50 days. 17. Gametophyte reniform with wide wings in *B. portoricensis*, 50 days.

Rhizoids develop over the ventral surface near the cushion region and are present from the earliest developmental stages.

Vegetative propagation is present in old thalli 6–8 months after sowing. In *Bolbitis portoricensis*, new gametophytes can develop from marginal cells, and by these means perpetuate the growth of the sexual generation.

The gametangia are characteristic of typical leptosporangiate ferns and the antheridia develop before the archegonia (Figs. 22–28). The antheridium is hemispherical, slightly flattened dorsi-ventrally and composed of three cells: a discoidal basal cell, a cylindrical central cell, and a lid-shaped opercular cell. These three cells surround the spermatogenous mass; during dehiscence, the opercular cells break off or collapse, liberating the antherozoids. Antheridia frequently grow among the tangled rhizoids of mature thallus. These antheridia develop superficially and are irregularly distributed over the entire lower surface, on the wings, and on the sides of the cushion. They develop in *B. bernoullii*, *B. portoricensis* and *B. umbrosa* from day 79 to 121 (Figs. 22–24, 26–28).

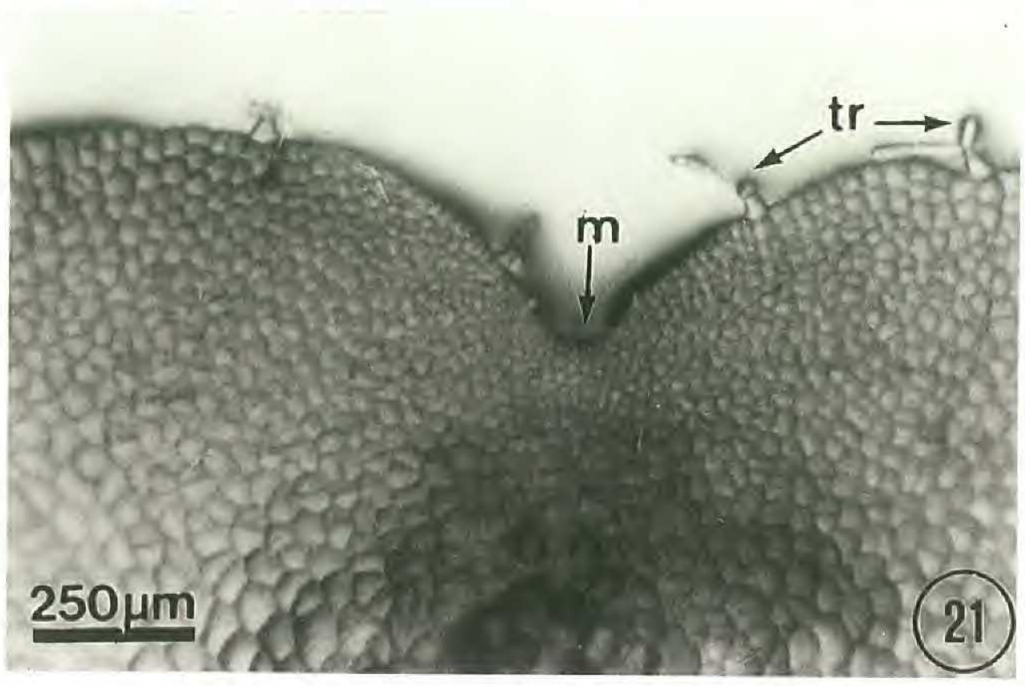
Archegonia generally develop one or two weeks after the antheridia. Archegonia are restricted to the cushion and to the area surrounding the notch. They have elongate necks that curve towards the basal region of the gametophyte. The neck canal cell is single, bi-nucleate and, near maturity, conspicuously swollen. The mouth has the four characteristic opercular cells. In *Bolbitis portoricensis* archegonia develop between day 62 and 79, in *B. bernoullii* by day 79, and in *B. umbrosa* from days 71 to 86 (Figs. 29–31).

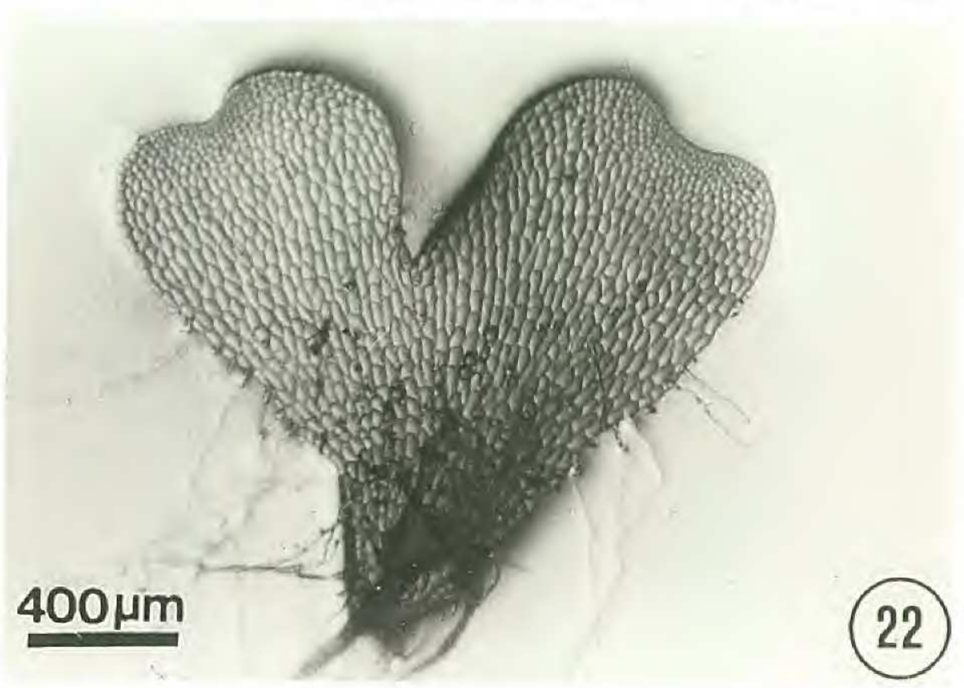
Sporophytes developed only in *Bolbitis portoricensis*, they appeared 6 to 8 months after spore germination (Fig. 32). The first juvenile leaf is shortly petiolate, simple, elongate and has a solitary or bifurcated vascular supply and a few unicellular, papillate hairs distributed along the blade. The lamina has polocytic-type stomata, wherein the guard cells are joined at one extreme (pole) and surrounded by a subsidiary cell (Fig. 33). This type of stomata is found in many leptosporangiate ferns including the Lomariopsidaceae. Their size varies from 35 to 65 μm (Van Cotthem, 1968, Hennipman, 1977). *Bolbitis portoricensis* clearly showed epidermal cells with undulated contours (see Fig. 33).

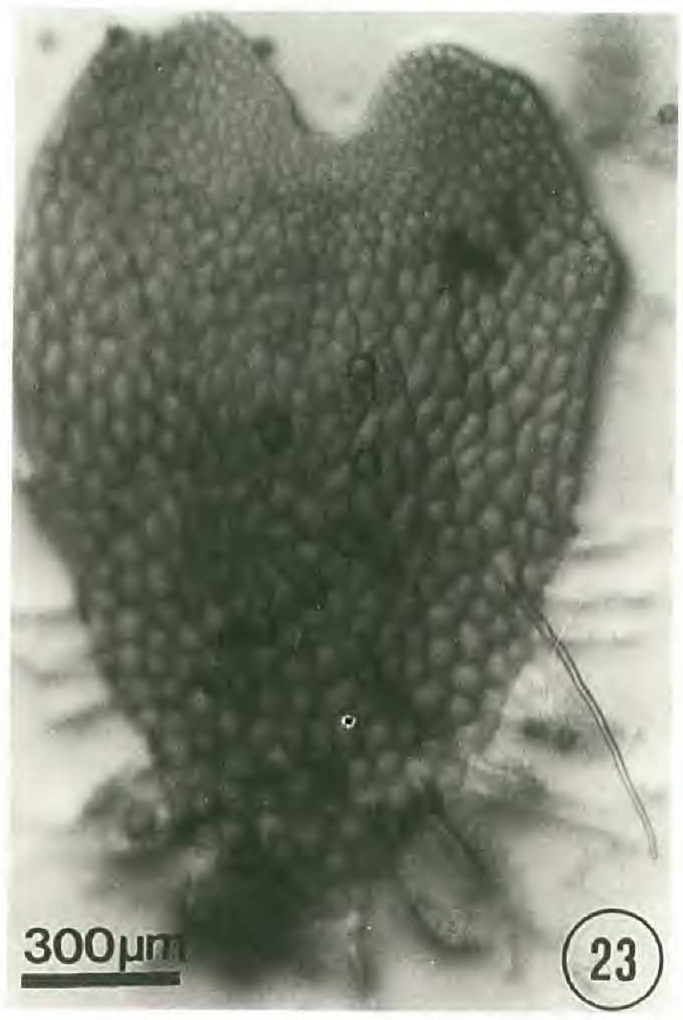
Discussion

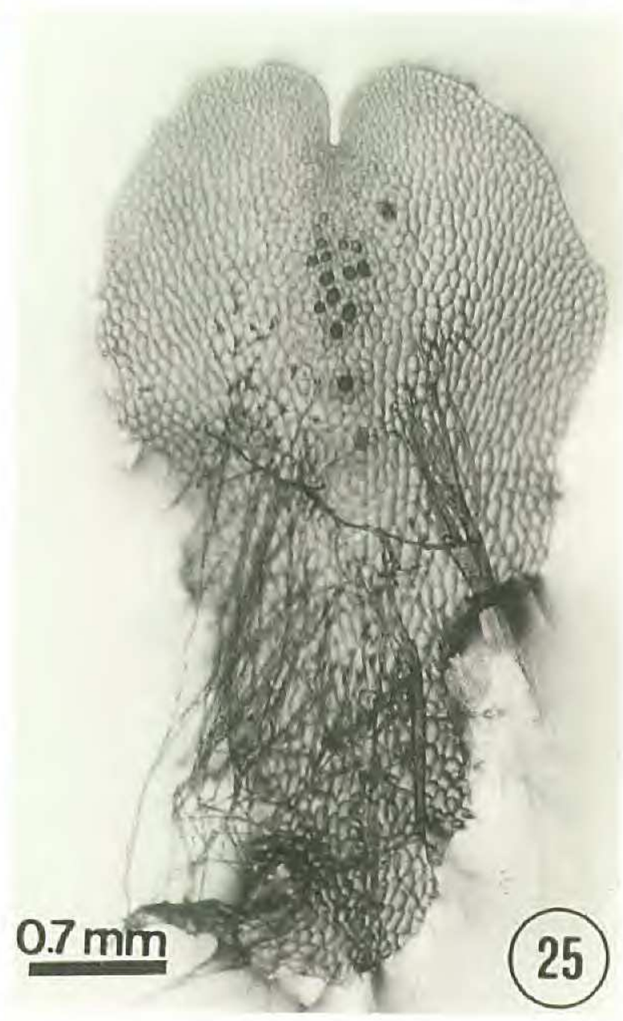
Our observations show that the development of the gametophyte in *Bolbitis bernoullii, B. portoricensis* and *B. umbrosa* is uniform. The few differences noted were insignificant and, fundamentally, all can be associated with time of development: the germination times of three species are asynchronous. Similarities found include monolete spores, *Vittaria*-type germination, *Drynaria*-type prothallial development, and gametangia of the advanced type for leptosporangiate ferns. Spore size in *Bolbitis* was found to be in agreement with data given by Hennipman (1970, 1977).

We found several differences. The spores are a little larger in *Bolbitis bernoullii* than in *B. portoricensis* and *B. umbrosa*. *Bolbitis bernoullii* has









FIGS. 18–25. Adult gametophytes of *Bolbitis*. 18. Branched gametophyte in *B. portoricensis*, 90 days. 19. Cordiform-spatulate gametophyte with hairs in *B. portoricensis*, 52 days. 20. Marginal hair in *B. portoricensis*, 52 days. 21. Meristematic zone with pluricellular and marginal branched hairs in *B. portoricensis*, 107 days. 22. Gametophytes with a deep notch in *B. portoricensis*,

a wider perine than *B. portoricensis*. The germinative filaments are short, 5 to 7 cells, in *B. umbrosa*, and longer, 7 to 14 cells, in *B. bernoullii* and *B. portoricensis*. There is also variation in the adult shapes of the gametophytes. The cushion is not well defined in *B. bernoullii*, but is in the other two species. Antheridia develop between day 79 and day 121 in all three species, and the archegonia appear between days 62 and 79 in *B. portoricensis* and between days 71 and 86 in *B. bernoullii* and *B. umbrosa*.

Nayar and Kaur (1964a) reported the presence of collenchyma-like thickenings on the corners and lateral walls of cells in the gametophytes wings in *B. crispatula, B. costata* and *B. subcrenata*. Such thickenings were absent however, in the species studied here. This special type of thickening in walls of prothallial cells is characteristic of *Anemia* (Schizaeaceae; Stokey, 1951) and is uncommon in other fern genera.

Bolbitis shares many gametophytic and sporophytic characteristics with *Egenolfia* and *Elaphoglossum* as well as other genera of the Lomariopsidaceae. These similarities include monolete spores with a prominent perine, *Vittaria*-type germination, *Drynaria*-type development, and advanced gametangia form. Differences occur in the shape of the adult gametophyte, which can be cordate or ribbon-shaped, in the presence of hairs, longevity and growth rate.

Although hairs in the gametophytic and sporophytic phases are used as taxonomic tools, our current knowledge is insufficient to assess their taxonomic value in this group (Nayar, 1956). Nayar and Kaur (1965b) statements regarding the presence of unicellular, marginal, papillate, non-glandular scattered hairs, could refer to their presence on gametophytes of *B. heteroclita*. Unicellular hairs with an extracellular waxy secretion mentioned (Nayar and Kaur, 1965b) for *B. presliana* but this needs to be confirmed. The prothallus in *Bolbitis* is naked during early phases of development. Two- to three-celled uniseriate hairs, with swollen terminal cells, are produced near the prothallial apex in *B. subcrenata* and *B. semicordata*. Each hair has a 1- to 4-cell long stalk, with a swollen, brown (from tannins) terminal cell that gives it a bulbous aspect, as *B. heteroclita* and *B. repanda* (Nayar and Kaur, 1971, Atkinson 1973). These hairs have not been seen in our species.

Hairs generally develop when the prothallial lamina becomes cordiform; they are present in vegetative and adult forms. We have seen sparse bicellular, short marginal hairs located along the sides of the meristematic notch, and dispersed on the wings, and the anterior part of the gametophyte in *B. bernoullii*. Such hairs are not glandular, have sparse chloroplasts and lack the swollen terminal cells as seen in *B. umbrosa* (day 50) and in *B. portoricensis* (day 62). Stokey and Atkinson (1957, 1964) mention glabrous gametophytes in *Bolbitis*.

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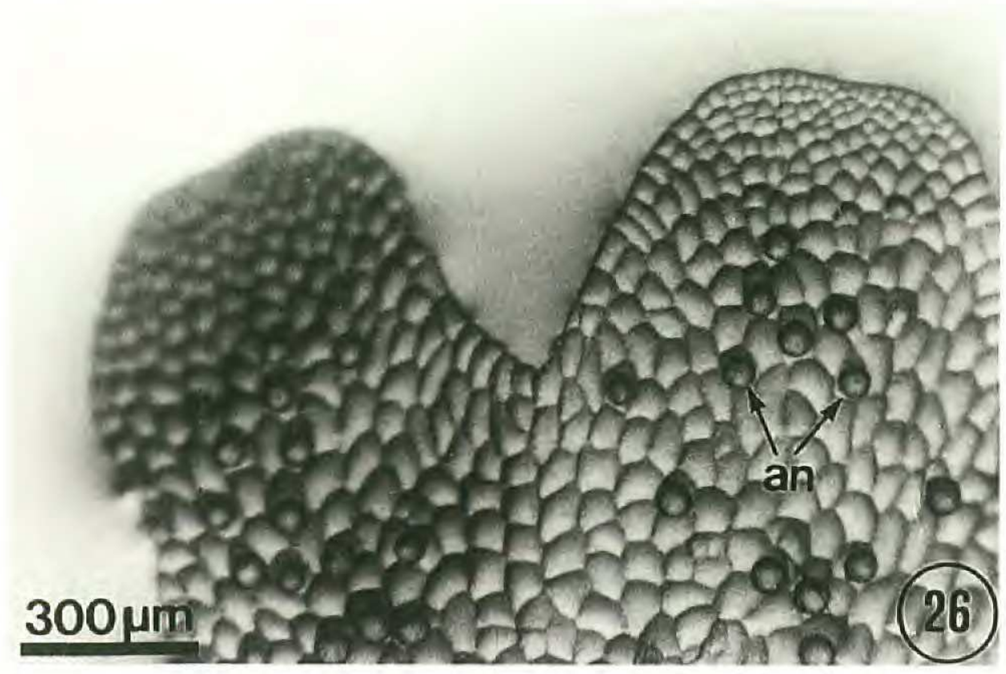
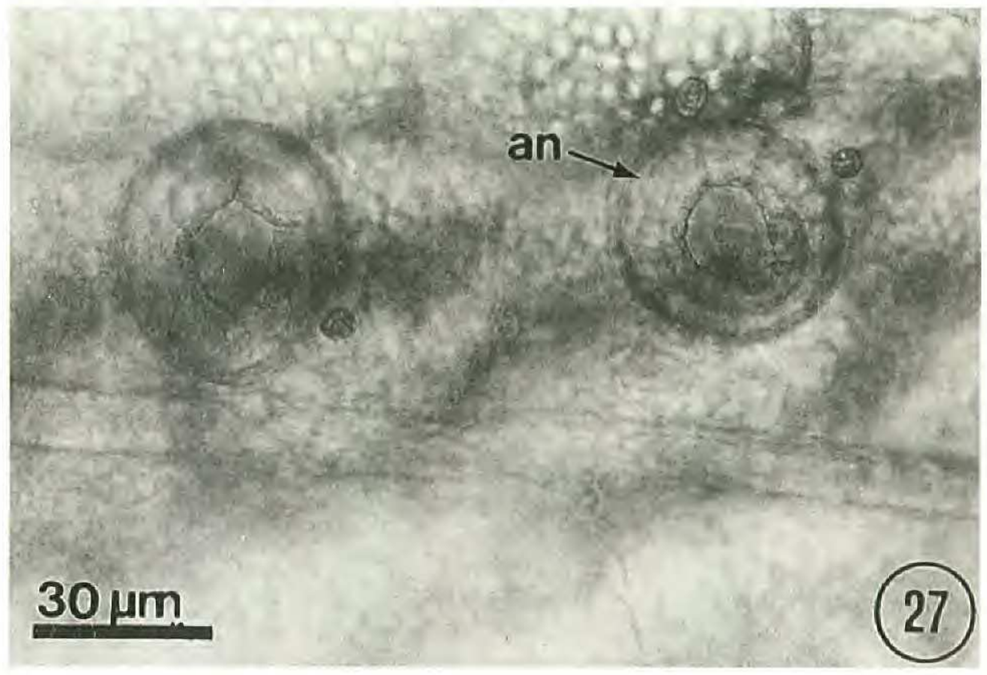
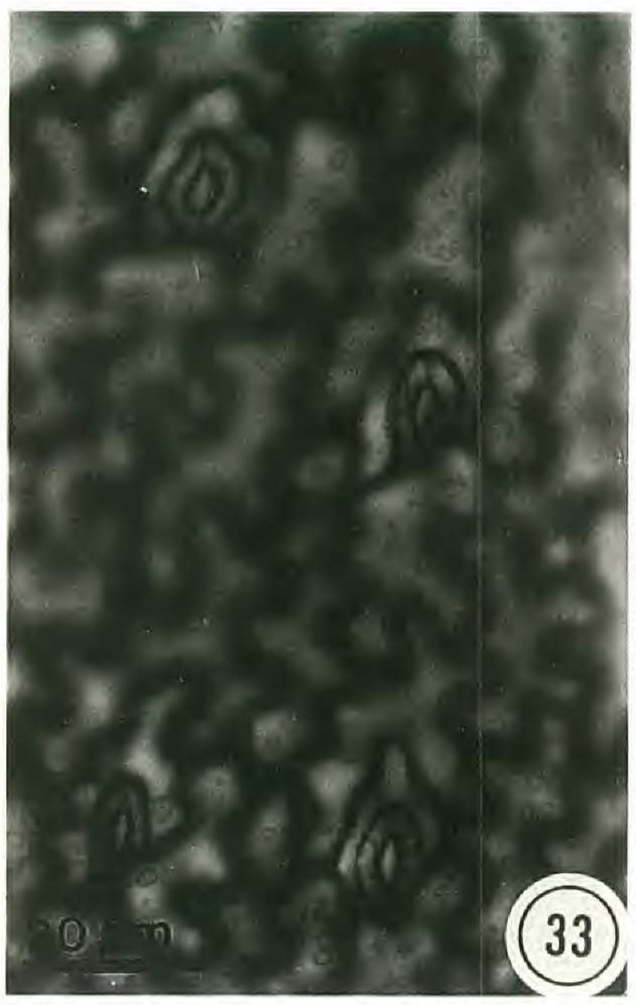
50 days. 23. Gametophyte with antheridia in *B. bernoullii*, 79 days. 24. Spatulate gametophyte with antheridia in *B. umbrosa*, 78–79 days. 25. Spatulate gametophyte with archegonia in *B. portoricensis*, 78–91 days. m= meristematic zone, tr= hair.







FIGS. 26–33. Gametangia and sporophytes of *Bolbitis*. 26. Antheridia on the wings in *B. portoricensis*, 79 days. 27. Antheridia in *B. portoricensis*, 91–95 days. 28. Antheridia in *B. umbrosa*, 79–86 days. 29. Archegonia on the cushion in *B. portoricensis*, 78 days. 30–31. Mouths of archegonia in *B. portoricensis*, 79–91 days. 32. First leaves of the sporophyte in *B. portoricensis*, 150 days. 33. Polocytic stomata in *B. portoricensis*, 150 days. An= antheridium, ar= archegonium, oc= opercular cell, ma= mouth of archegonium, mc= medial cell.

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Table of Contents for Volume 96

(A list of articles arranged alphabetically by author)